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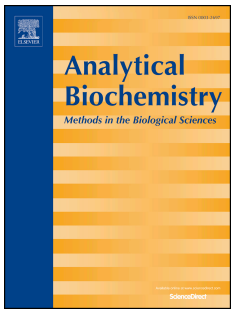

RPA using a multiplexed cartridge for low cost point of care diagnostics in the field

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RPA Using a Multiplexed Cartridge for Low Cost Point of Care Diagnostics in the Field

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Abstract

A point of care device utilising Lab-on-a-Chip technologies that is applicable for biological pathogens was designed, fabricated and tested showing sample in to answer out capabilities. The purpose of the design was to develop a cartridge with the capability to perform nucleic acid extraction and purification from a sample using a chitosan membrane at an acidic pH. Waste was stored within the cartridge with the use of sodium polyacrylate to solidify or gelate the sample in a single chamber. Nucleic acid elution was conducted using the RPA amplification reagents (alkaline pH). Passive valves were used to regulate the fluid flow and a multiplexer was designed to distribute the fluid into six microchambers for amplification reactions. Cartridges were produced using soft lithography of silicone from 3D printed molds, bonded to glass substrates. The isothermal technique, RPA is employed for amplification. This paper shows the results from two separate experiments: the first using the RPA control nucleic acid, the second showing successful amplification from *Chlamydia Trachomatis*.

Endpoint analysis conducted for the RPA analysis was gel electrophoresis that showed 143 base pair DNA was amplified successfully for positive samples whilst negative samples did not show amplification. End point analysis for *Chlamydia Trachomatis* samples was fluorescence detection that showed successful detection of 1 copy/µL and 10 copies/µL spiked in a MES buffer.

1. Introduction

Point of care (POC) diagnostics for molecular diagnostics have been the focus of many research projects since the beginning of the century. Whilst the GeneXpert has seen success, the four module platform has never been seen as truly point of care. This has changed with the advent of the GeneXpert Omni – a portable battery powered POC diagnostic; however the limitation to run a single diagnostic in one run is a limitation and benchtop sample preparation is still required [1]. POC devices such as the GeneXpert use nucleic acid (NA) amplification for the detection of pathogens. This paper focuses on the amplification of double stranded nucleic acid, deoxyribonucleic acid (DNA) found in bacteria. The GeneXpert uses the most common method for NA detection, polymerase chain reaction (PCR) [2]. PCR requires thermal cycling through three reaction temperatures 95, 50 and 72 $^{\circ}$C. Two primers are required for PCR, nucleic acid is doubled in each thermal cycle, overall the reaction is slower than isothermal equivalents and the requirement for thermal cycling has made it a less popular solution at the point of care due to the high power requirements [3, 4]. Fluidic devices have been developed incorporating PCR using fluorescence markers, capillary gel electrophoresis and DNA microarrays with volumes ranging from 4-50 µL [4].